Supplementary information

Fig. S1. Standard RT-PCR using stem-loop primer is not capable of distinguishing 3′-variants of miRNA

(A) Schematic representation of a standard RT-PCR using a stem-loop RT primer for miRNA quantification (37). A stem-loop RT primer was specifically hybridized to the 3′-end of the target RNA, followed by reverse transcription. The resultant cDNA was amplified and quantified by Real-time PCR using forward and reverse primers derived from the targeted miRNA and RT primer, respectively.

(B) The standard RT-PCR was designed to target miR-16, which was applied for the detection of synthetic miR-16 and its 3′-variants whose sequences are shown above the graph. The method was performed as previously reported (37) with minor modifications. For reverse transcription, synthetic miR-16 or its 3′-variants (200 amol) was incubated in the reaction mixture (20 μL volume) containing SuperScript III Reverse Transcriptase (Life Technologies) and 1 pmol of stem-loop RT primer (5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCCAA-3′) at 16°C for 30 min, followed by incubation at 42°C for 30 min. The resultant cDNA solution was diluted to 1:5, and 1.5 μL of this solution was used for the Real-time PCR reaction (10 μL volume) using SsoFast EvaGreen Supermix (BioRad) and StepOne Plus Real-time PCR machine (Applied Biosystems). The miR-16 and its 3′-variants were similarly detected by the method, indicating that the method does not have a resolution to distinguish target RNA from its 3′-variants.

Fig. S2. Schematic representation of the quantification of miR-16 by 3′-Db-PCR

Fig. S3. 5′-Db-PCR, 3′-Db-PCR, and Db-PCR amplified cDNAs as a clear band

The amplified cDNAs resulting from 5′-Db-PCR (5′-Db), 3′-Db-PCR (3′-Db), and Db-PCR (Db) for detection of miR-16 and miR-21 in HeLa total RNA were developed by 3% Metaphor gel electrophoresis (LONZA). The expected band sizes from the 5′-Db-PCR, 3′-Db-PCR, and Db-PCR are 59, 49, and 66 bp, respectively; a clear band with the expected size was observed in all lanes.

Fig. S4. Proportional correlation of miR-16 input to the Ct value obtained by 3′-Db-PCR, 5′-Db-PCR, and Db-PCR

Synthetic miR-16 (0.0156, 0.0625, 0.125, 0.25, and 1 fmol) were quantified by 3′-Db-PCR, 5′-Db-PCR, and Db-PCR. Each data set represents the average of three independent experiments with bars showing the SD.

Fig. S5. Sequences and secondary structures of the 3′-Db-adapter and targeted *Bombyx* piRNAs quantified by 3′-Db-PCR

Fig. S6. Schematic representation of the quantification of miR-16 by 5′-Db-PCR

Fig. S7. Sequences and secondary structures of the 5′-Db-adapter and targeted human microRNAs quantified by 5′-Db-PCR

Fig. S8. Schematic representation of the quantification of miR-16 by Db-PCR

Fig. S9. Sequences and secondary structures of the adapters and targeted small RNAs quantified by Db-PCR

Supplementary Table S1. Sequences of adaptors and primers for 3′-Db-PCR

Target	Primer/adapter	Sequence (5′–3′)
miR-16	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGCGCCAA
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	GCGCTAGCAGCACGTAAATAT
	TaqMan probe	/56-FAM/TGGCGCTCA/ZEN/GTG/3IABkFQ/
piR-1	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGGTTCGA
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	TCAAAAACTAACGGATTG
	TaqMan probe	/56-FAM/TCGAACCTC/ZEN/AGTGCAG/3IABkFQ/
piR-1-[3′+AGUC]	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGGACTGT
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	TCAAAAACTAACGGATTG
	TaqMan probe	/56-FAM/AACAGTCCT/ZEN/CAGTGCAG/3IABkFQ/
piR-2	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGCCGCAG
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	AAAAGCATGAGAATTTGC
	TaqMan probe	/56-FAM/CTGCGGCTC/ZEN/AGTGCA/3IABkFQ/
piR-2-[3′+ACCA]	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGTGGTCC
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	AAAAGCATGAGAATTTGC
	TaqMan probe	/56-FAM/CGGACCACT/ZEN/CAGTGCAG/3IABkFQ/

Supplementary Table S2. Sequences of adaptors and primers for 5′-Db-PCR

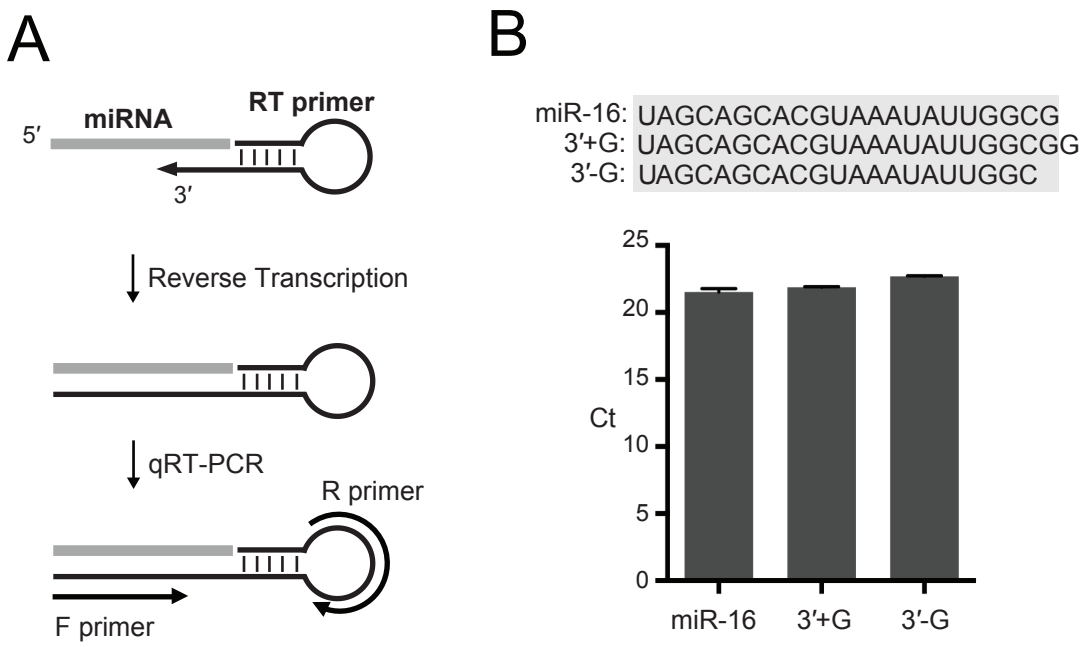
Target	Primer/adapter	Sequence (5′–3′)
miR-16	5′-Db-adapter	CTGCTACTCAGTGCGTGGGAGGGTGTGTGGTCTTGCTTGGTGTGCACTGrArG
	RT primer	GCGACGCCAATATTTACGTG
	Reverse primer	GCGACGCCAATATTTACGTG
	Forward primer	GAGGGTGTGTGGTCTT
	TaqMan probe	/56-FAM/TGTGCACTG/ZEN/AGTAGCAG/3IABkFQ/
miR-26a	5′-Db-adapter	CTTGAACTCAGTGCGTGGGAGGGTGTGTGGTCTTGCTTGGTGTGCACTGrArG
	RT primer	CAGCCTATCCTGGATTA
	Reverse primer	CAGCCTATCCTGGATTA
	Forward primer	GAGGGTGTGTGGTCTT
	TaqMan probe	/56-FAM/TGTGCACTG/ZEN/AGTTCAAG/3IABkFQ/
miR-26a-[5′+A]	5′-Db-adapter	TTGAATCTCAGTGCGTGGGAGGGTGTGTGGTCTTGCTTGGTGTGCACTGrArG
	RT primer	CAGCCTATCCTGGATTA
	Reverse primer	CAGCCTATCCTGGATTA
	Forward primer	GAGGGTGTGTGGTCTT
	TaqMan probe	/56-FAM/TGTGCACTG/ZEN/AGATTCAA/3IABkFQ/

A, G, C, and T designate DNA, whereas rA and rG designate RNA.

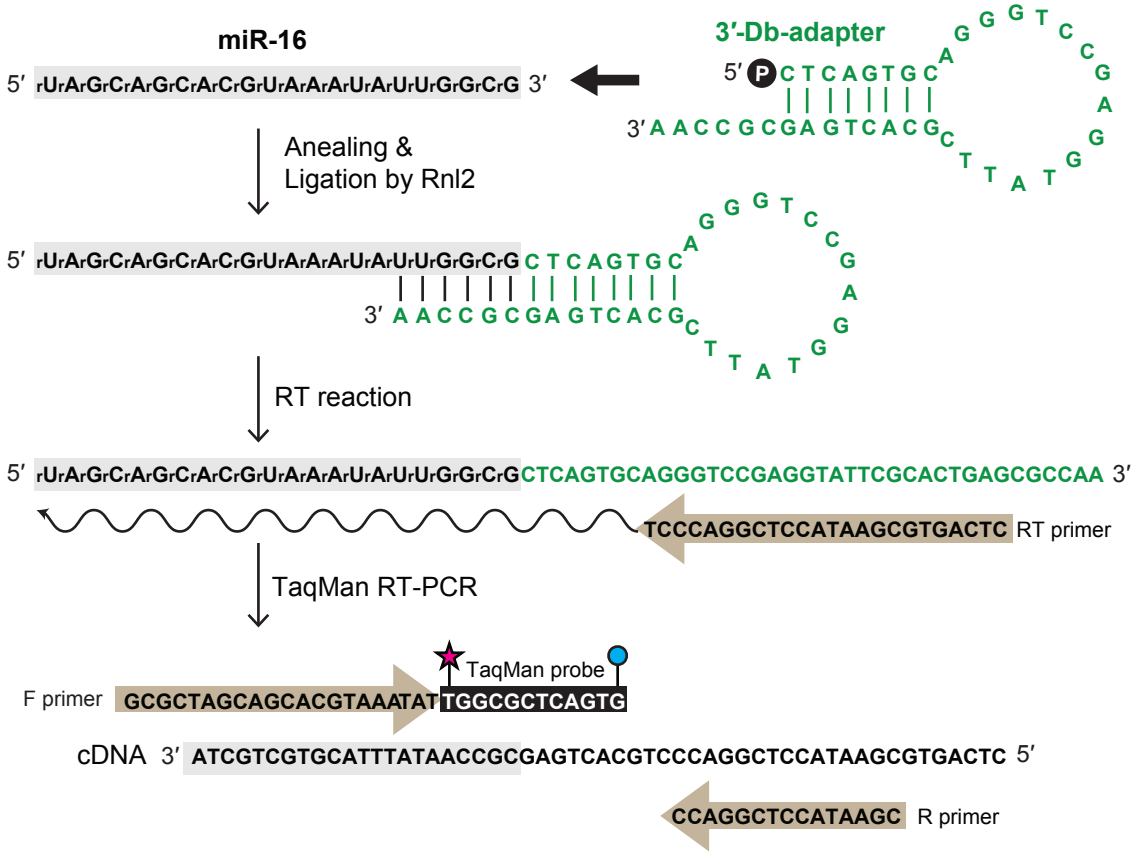
Supplementary Table S3. Sequences of adaptors and primers for Db-PCR

Target	Primer/adapter	Sequence (5′–3′)
miR-16	5′-Dbs-adapter-6	CTGCTACGTCG/idSp/TGGAGTGTGTGCTTTGArCrG
	5′-Dbs-adapter-12	TACGTGCTGCTACGTCG/idSp/TGGAGTGTGTGCTTTGArCrG
	5′-Dbs-adapter-16	TATTTACGTGCTGCTACGTCG/idSp/TGGAGTGTGTGCTTTGArCrG
	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGCGCCAA
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	TGGAGTGTGTGCTTTGACGTAGC
	TaqMan probe	/56-FAM/AAATATTGG/ZEN/CGCTCAGTGCA/3IABkFQ/
miR-21	5′-Dbs-adapter	TCAGTCTGATAAGCTACGTCG/idSp/TGGAGTGTGTGCTTTGArCrG
	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGTCAACA
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	TGGAGTGTGTGCTTTGACGTAGC
	TaqMan probe	/56-FAM/AGACTGATG/ZEN/TTGCTCAGTGCA/3IABkFQ/
piR-2	5′-Dbs-adapter	AAATTCTCATGCTTTTCGTCG/idSp/TGGAGTGTGTGCTTTGArCrG
	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGCCGCAG
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	TGGAGTGTGTGCTTTGACGAAAAG
	TaqMan probe	/56-FAM/CTGCGGCTC/ZEN/AGTGCA/3IABkFQ/
piR-2-[3′+ACCA]	5′-Dbs-adapter	AAATTCTCATGCTTTTCGTCG/idSp/TGGAGTGTGTGCTTTGArCrG
	3′-Db-adapter	/5Phos/CTCAGTGCAGGGTCCGAGGTATTCGCACTGAGTGGTCC
	RT primer	CTCAGTGCGAATACCTCGGACCCT
	Reverse primer	CGAATACCTCGGACC
	Forward primer	TGGAGTGTGTGCTTTGACGAAAAG
	TaqMan probe	/56-FAM/CGGACCACT/ZEN/CAGTGCAG/3IABkFQ/

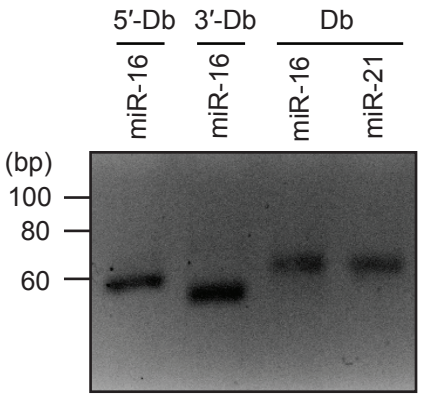
A, G, C, and T designate DNA, whereas rC and rG designate RNA. 5′-Db-adapter-6, 5′-Db-adapter-12, and 5′-Db-adapter-16 designate a 5′-Db-adapter with 6, 12 and 16 nt protruding 5′-end, respectively.



Honda et al. Figure S1



Honda et al. Figure S2



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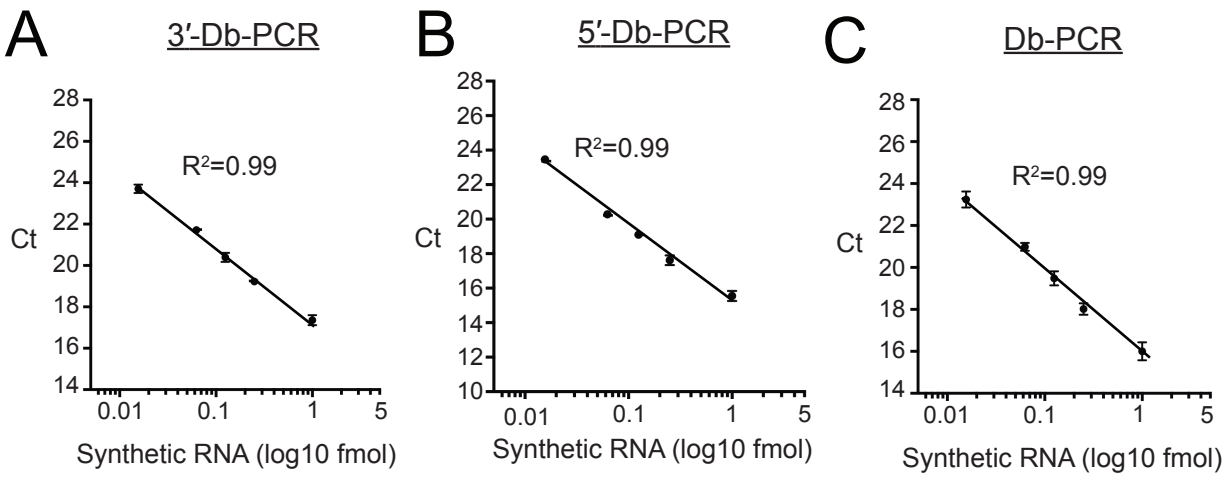
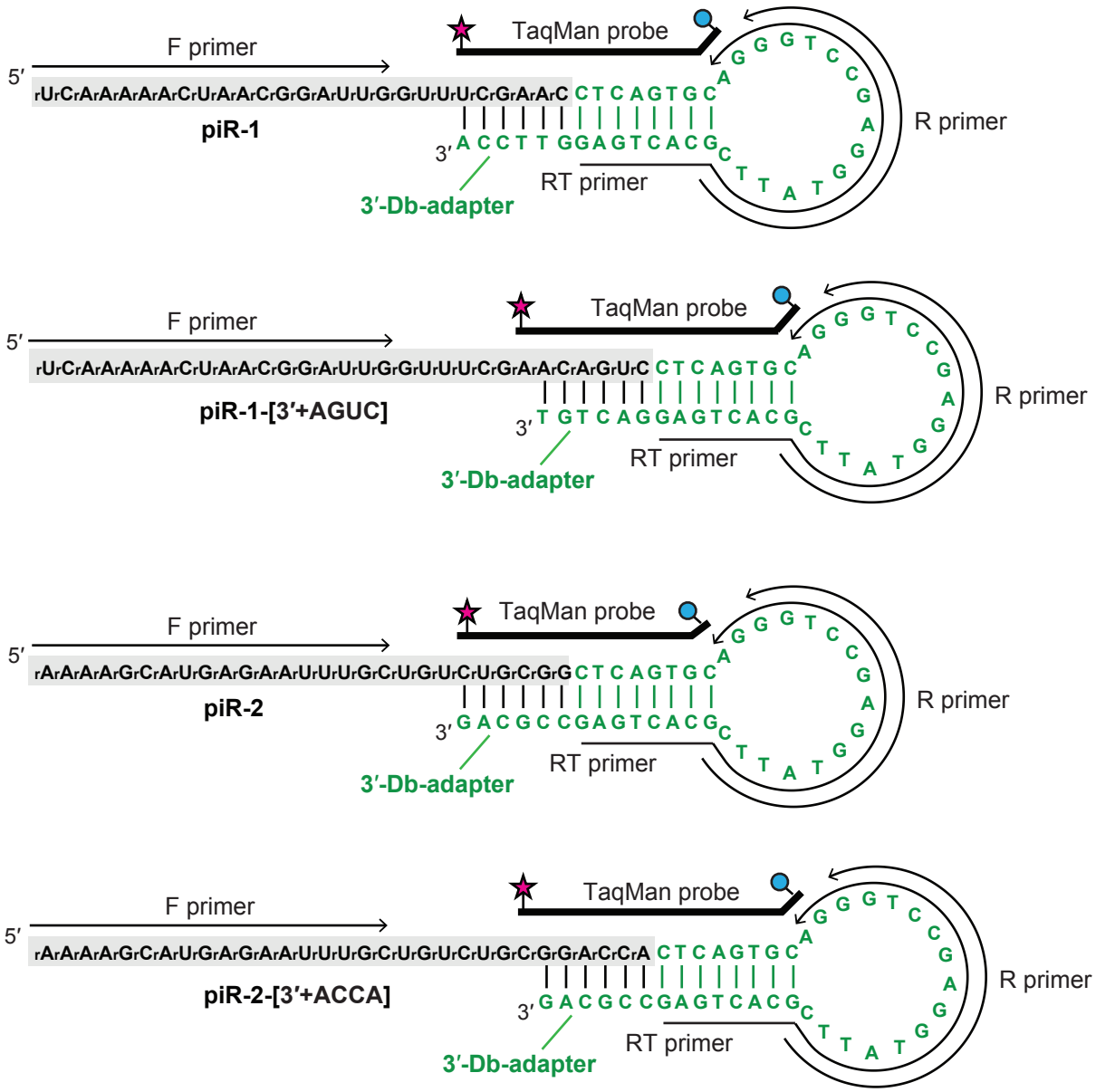


Honda et al. Figure S4



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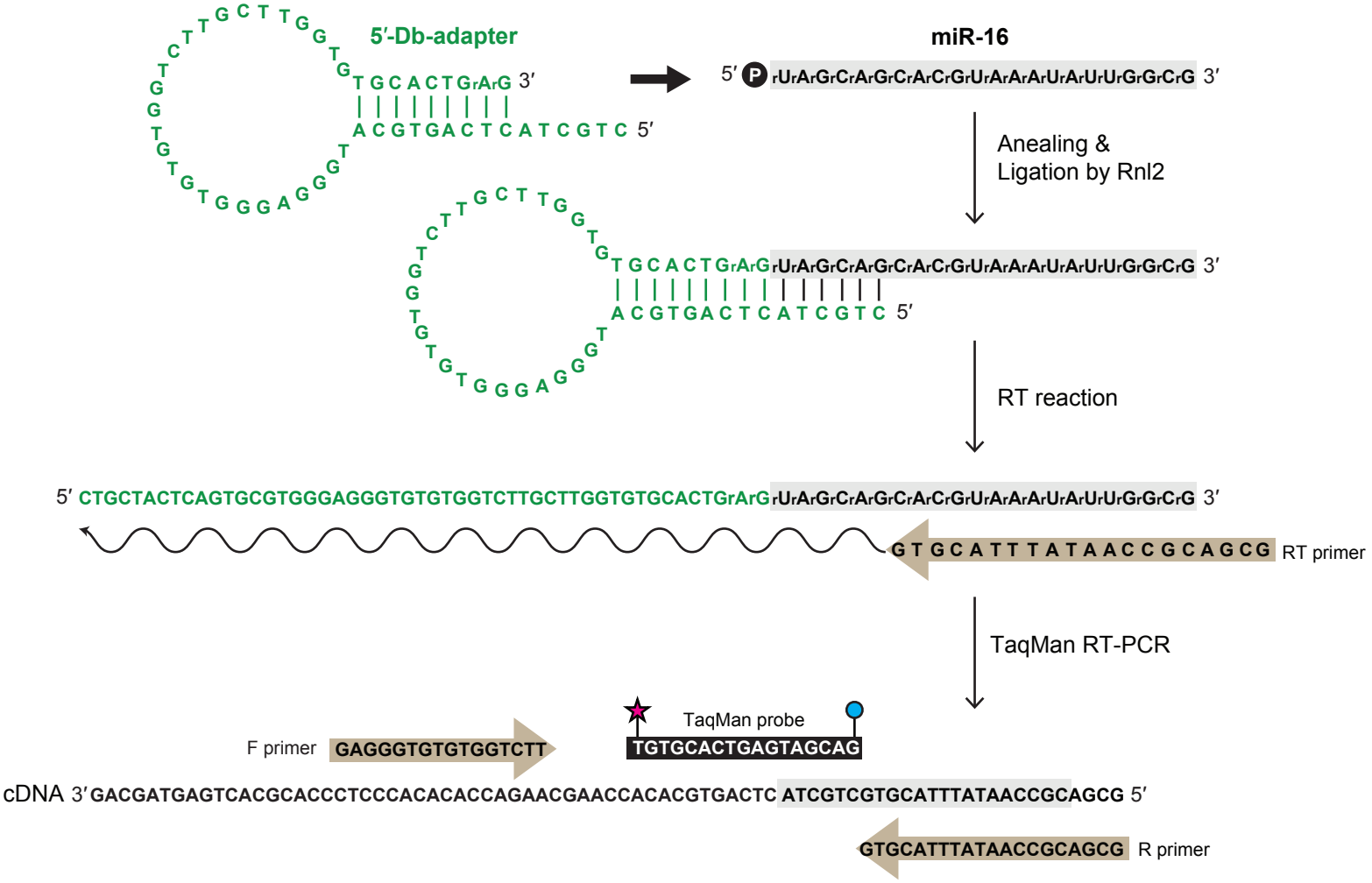

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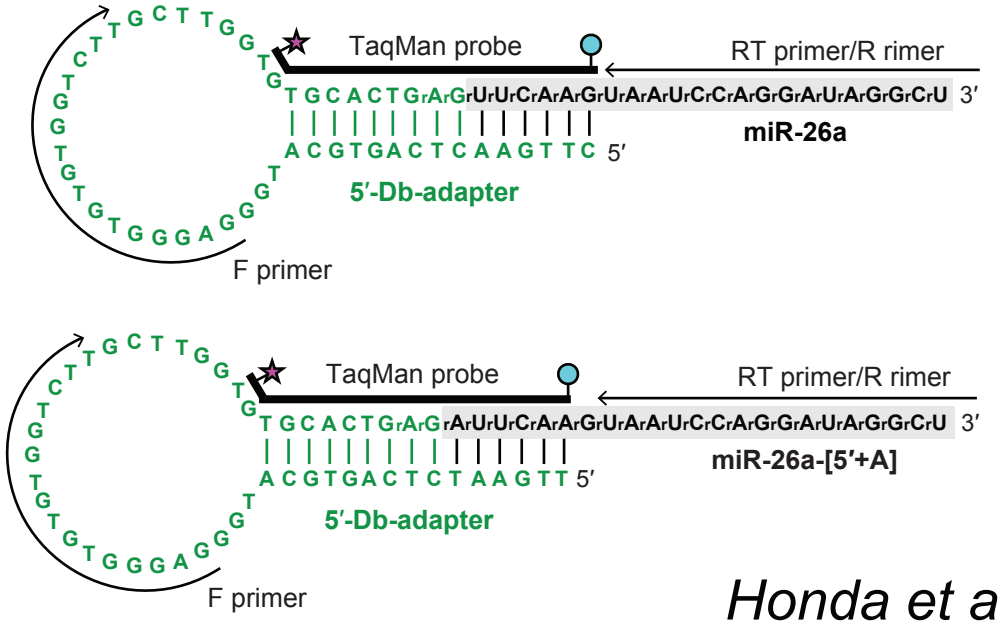

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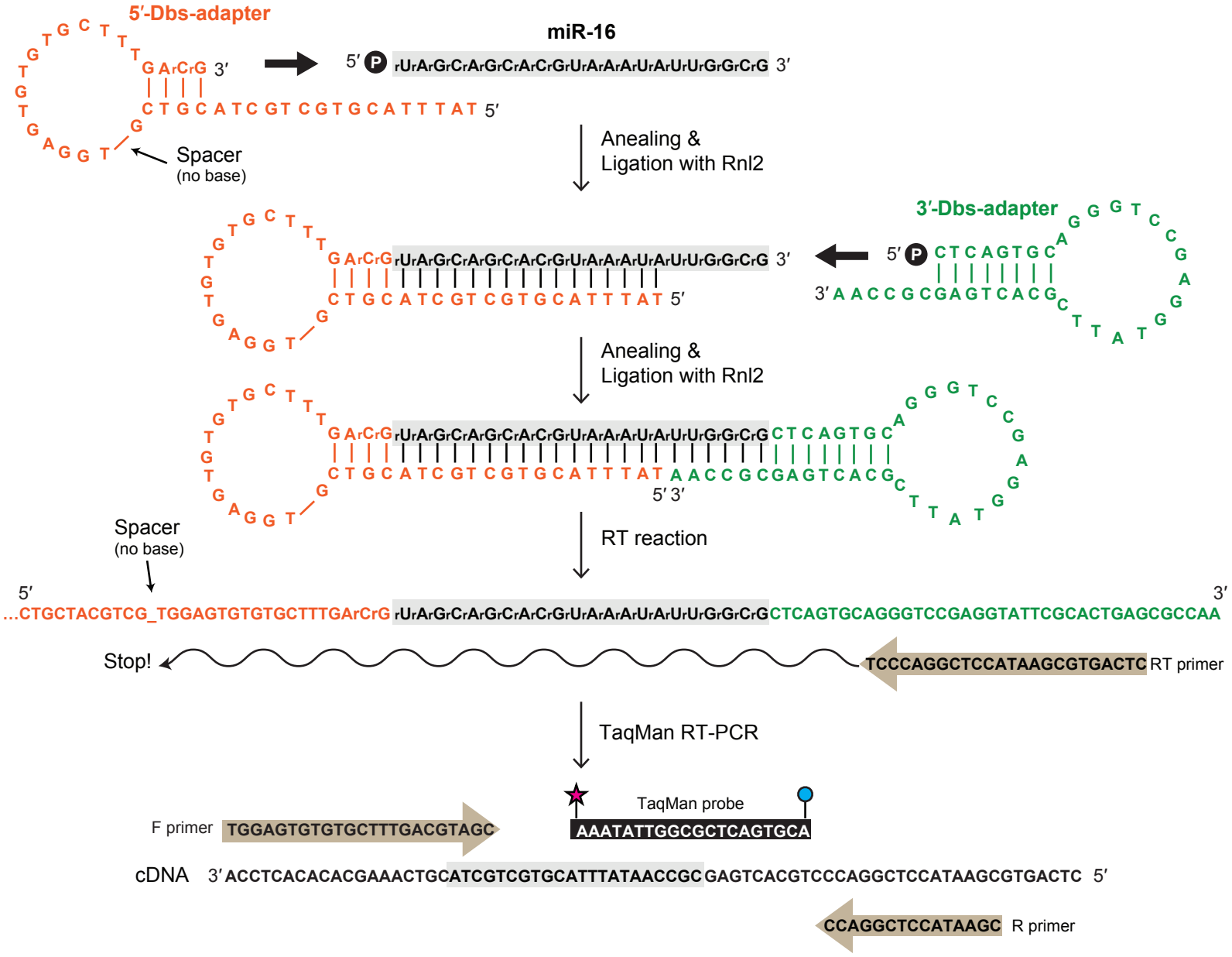
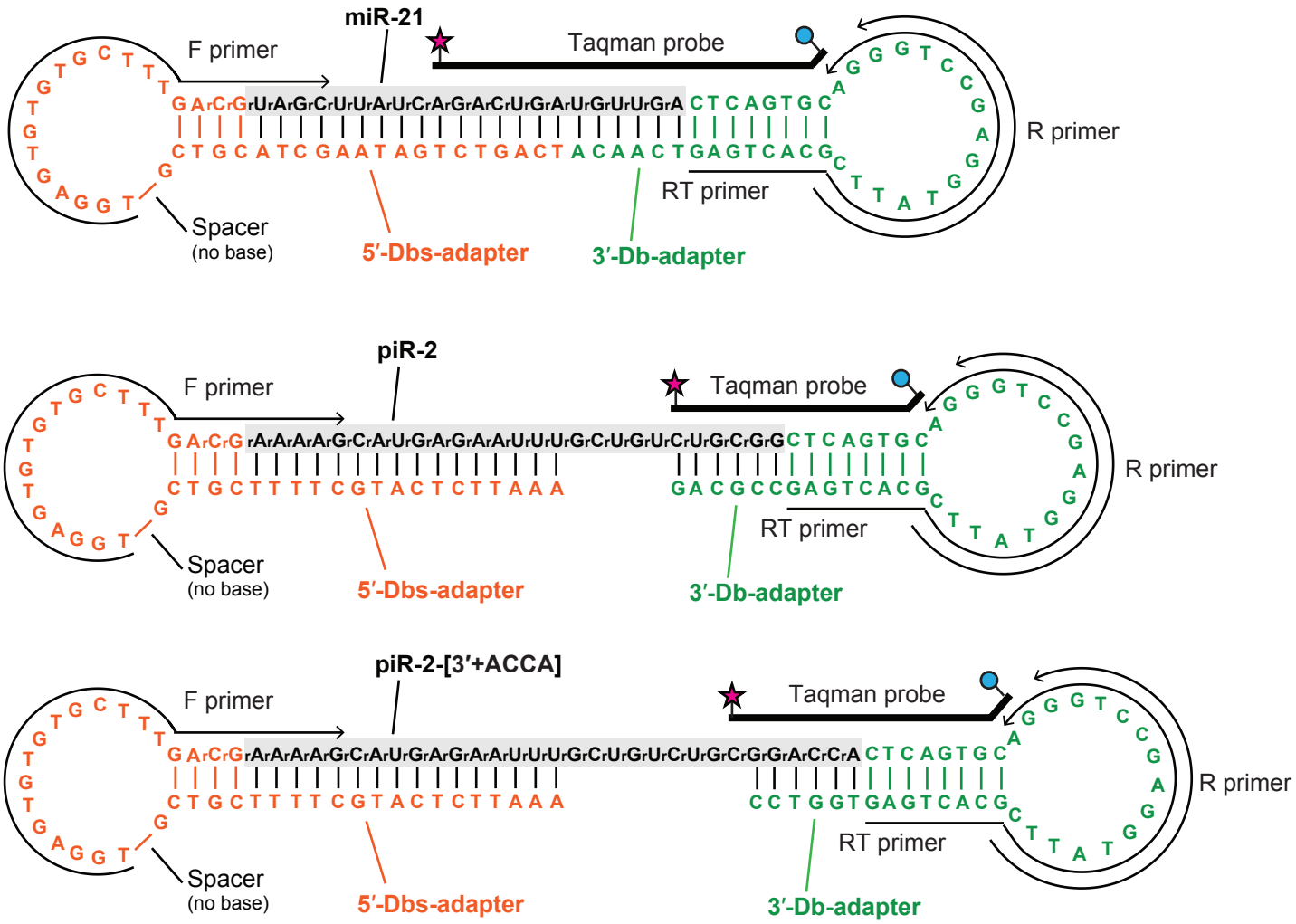


Honda et al. Figure S8



Honda et al. Figure S9